during a 30 second cycle is maintained within plus or minus 1°C. Support is found at page 35, lines 5-9.

Claim 39 is amended to state that the cooling step is done at a rate of at least about 0.5°C per second. Support is found at page 42, lines 1-5.

New Claim 57 is added as a dependent claim from Claim 30, wherein the repeating of steps c through f is completed in 30 to 60 seconds. Support is found at page 23, lines 18-21.

New Claim 58 is added as a dependent claim to any one of Claims 30-35, wherein sad sample holding structure is a capillary vessel.

Rejections under 35 U.S.C. § 112, first paragraph

Claims 30-45 are rejected under 35 U.S.C. § 112, first paragraph as containing subject matter not described in the specification so as to reasonably convey that Applicants had possession of the claimed invention. Applicants respectfully traverse.

The standard for determining whether a claim is in compliance with the description requirement is discussed at MPEP § 2163.02. "[T]he fundamental factual inquiry is whether a claim defines an invention that is clearly conveyed to those skilled in the art at the time the application was filed." This section also notes that "[t]he subject matter need not be described literally (i.e., using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement." Also relevant to the present rejection regarding numerical range limitations, "the analysis must take into account which ranges one of ordinary skill in the art would consider inherently supported by the discussion in the original disclosure." (MPEP § 2163.05(III)) Finally, MPEP § 608 explains, "Applicant may rely for disclosure upon the specification with original claims and drawings, as filed." (emphasis added)

The Office Action cites the phrase "completed in approximately 60 seconds or less" in Claim 30, "60 seconds or less" in Claim 35 and, presumably, "less than 60 seconds" in Claims 40 and 45 as new material. Each of these claims has been amended to state a time range for completion that is fully supported by the disclosure.

Applicants note that "steps c through f" of Claim 30 and the "heating and cooling step" of Claim 35 each refer to a thermal cycle, as do Claims 40 and 45. The specification

states that 15 or 30 of these cycles can be carried out in 30, 15, 10 to 5 or fewer minutes (page 29, lines 8-12). Thirty cycles in five minutes works out to 1 cycle in 10 seconds. Thirty cycles in 30 minutes (or 15 in 15 minutes) works out to 1 cycle in 60 seconds. Fifteen cycles in 30 minutes works out to 1 cycle in 120 seconds. Clearly, all of the ranges of the amended claims are supported, if not literally, inherently.

The amended time ranges are further supported by the drawings, in particular Figures 9A-G. Plots C and D of Figure 9A disclose cycle times of 90 and 30 seconds, respectively. The plots of Figures 9B-G show cycle times ranging from about 11 seconds (9G) to more than 40 seconds (9C). Therefore, from the disclosure the ordinary artisan would certainly know that the Applicants were in possession of the claimed invention at the time of filing.

The Office action cites the phrases "less than one second" in Claim 36 (and presumably Claims 32, 34, 38, 42 and 44), "less than 5 seconds" in Claim 37 and "less than 32 seconds" in Claims 41 and 43 as new matter. The Examiner is directed to the figures, in particular figures 5-7 and 9A-G. Figure 5 depicts an optimized temperature versus time profile for a single thermal cycle, wherein annealing occurs at 50-55°C and denaturation at 90-92°C (*see* page 24, lines 18-21). At the very least, one can see that the holding times for annealing and denaturation are less than 5 (and 32) seconds. Figures 6 and 7 show results from experiments wherein the denaturation times (Fig. 6) and annealing times (Fig. 7) were varied. The figures clearly indicate that denaturation holding times in the range of 64 seconds to less than one second were obtained and annealing times of 80 seconds to less than one second were also achieved. Annealing and denaturation times of less than one second are shown in Plots C and D of Figure 9A. And annealing and denaturation times ranging from 5 seconds to less than 1 second are depicted in figures 9B-G. From these, an ordinary artisan would easily realize that the Applicants were in possession of the invention, as claimed, at the time of filing.

The Office action cites the cooling rate of 1.48°C per second, found in Claim 39, as being new matter. While not necessarily agreeing with this conclusion, the cooling rate has been amended to one that is literally disclosed in the specification.

The Office Action cites the maintenance of a sample temperature homogeneity "within plus or minus 1°C during heating and cooling steps", found in Claim 35, as being new matter. While not necessarily agreeing with this conclusion, the phrase has been

amended to conform with a literal disclosure in the specification.

Based on the amendments and the foregoing arguments, Applicants submit that Claims 30-45 satisfy the written description requirement of 35 U.S.C. § 112, first paragraph. Therefore, the Examiner is respectfully requested to withdraw this rejection.

Claims 36-45 are rejected under 35 U.S.C. § 112, first paragraph as not being enabled by the specification. Applicants respectfully traverse.

The test of enablement is whether, at the time of filing, the specification provides sufficient information for one of ordinary skill in the art to practice the claimed invention without undue experimentation. The satisfaction of the enablement requirement should consider at least the factors of: a) breadth of the claims; b) nature of the invention; c) state of the prior art; d) level of skill of the ordinary artisan; e) level of predictability in the art; f) amount of direction provided by the inventors; g) the existence of working examples; and h) the quantity of experimentation need to make or use the invention based on the content of the specification (*In re Wands* 858 F.2d 731, 735, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988; MPEP § 2164.01(a)). Applicants submit that the disclosure, knowledge and products available in the art, and skill of the ordinary artisan allow such an artisan to practice the claimed methods without undue experimentation.

The Office Action notes the absence of any mention of a specialized sample holding structure, such as a capillary tube, in the rejected claims. The Office Action goes on to assert that such a structure is critical to the present invention and is lacking in the prior art, as noted in Wittwer et al. (*see* below). Applicants submit that the specification is fully enabling for one of skill in the art to utilize any of a number of sample holding structures as may be useful in a given application of the claimed methods.

The Examiner's attention is directed to pages 32-33 of the specification. The disclosure teaches that numerous sample holding structures are suited to various applications. The specification goes on to teach that emphasis should be placed on keeping the sample volume small and the surface area of the sample holding structure large, together representing a small thermal mass. Capillary tubes are provided as a best mode, particularly for the application of thermal cycling for PCR amplification, as is illustrated in the examples. Further instruction is provided in that a preferred sample holding structure contain a volume

of from about 0.1 to 10,000 µl. One of ordinary skill in the art is certainly able to take this instruction into consideration for a given application and determine a suitable sample holding structure, which may or may not be a capillary vessel.

The Office Action discusses that the prior art does not provide a thermal cycler with the capabilities of the present invention. However, the claimed methods are not a product of utilizing capillary vessels with otherwise known art. The use of capillary tubes is provided as a preferred embodiment, but clearly any vessel that takes into consideration the teachings of the present disclosure is available and recognizable by one of skill in the art. The claimed methods are made possible by the teachings of the specification as a whole. Determination of an appropriate sample holding structure can be easily obtained by the skilled artisan without undue experimentation.

In light of the discussion above, Applicants submit that Claims 36-45 satisfy the enablement requirements of 35 U.S.C. § 112, first paragraph. Therefore, the Examiner is respectfully requested to withdraw this rejection.

Rejections under 35 U.S.C. § 102(b)

Claims 30, 31, 33, 35, 37, 39-41, 43 and 45 are rejected under 35 U.S.C. § 102(b) as being anticipated by Wittwer et al., Biotechniques 10(1): 76-83 (1991). Applicants respectfully traverse.

The Examiner is first directed to the Preliminary Amendment submitted with this continuation application on August 11, 1998. The present application claims priority to U.S. Application No. 07/534,029 (the '029 application) filed June 4, 1990. In this original parent application the methods and results of the Wittwer et al. reference are fully disclosed as Example 1 at page 16, line 13 to page 19, line 12. This text is found at page 24, line 6 to page 27, line 15 of the present application and appeared in all intervening priority documents. Furthermore, figures 2 and 4 of the Wittwer et al. reference are identical to figures 6 and 7, respectively, of the present application, which also appeared in the '029 application and all intervening priority documents. This disclosure fully supports the claimed methods in question.

The disclosure of the Wittwer et al. paper is the work of the present inventors and is

fully disclosed in the parent application to which the present application claims priority. The priority document predates the publication of the cited reference. Therefore, the Wittwer et al. reference is not prior art under 35 U.S.C. § 102.

For these reasons, the Wittwer et al. reference does not anticipate Claims 30, 31, 33, 35, 37, 39-41, 43 and 45. Therefore, the Examiner is respectfully requested to withdraw this 35 U.S.C. § 102(a) rejection.

Claims 30-45 are rejected under 35 U.S.C. § 102(b) as being anticipated by Swerdlow et al., Biotechniques 15(3): 512-519 (1993). Applicants respectfully traverse.

As discussed above, much of the relevant text of the present application first appeared in the '029 application. In addition, the present application claims priority to U.S. Application No. 07/815,966 (the '966 application) filed January 2, 1992. With special regard to the present rejection, the text at page of 40, line 5 to page 41, line 12 and figure 9A-G appeared in the '966 application, as well as in each intervening priority document. Both the '029 and the '966 applications predate Swerdlow et al.

The Examiner states that the Swerdlow et al. reference discloses the same reaction as the Wittwer et al. reference (and the '029 application), but additionally discloses denaturation and annealing hold times of less than 1 second. Applicants first point out that figures 6 and 7, which appeared in the '029 application, show denaturation and annealing hold times, respectively, of less than 1 second (lane 1 of figure 6, lanes 1 and 2 of figure 7). In addition, the specification at page 41, lines 3-5, states that yield and product specificity are optimal when denaturation and annealing times are less than 1 second. Finally, figure 9A, plot D, and figure 9F clearly show denaturation and annealing times of less than 1 second.

Because of the disclosures in the '029 and '966 applications, to which the present application claims priority, and the disclosures which predate the cited references support the present claims, Claims 30-45 are not anticipated by Swerdlow et al. Therefore, the Examiner is respectfully requested to withdraw this rejection.

Rejections under 35 U.S.C. § 103(a)

Claims 30, 31, 33, 35, 37, 39-41, 43 and 45 are rejected under 35 U.S.C. § 103(a) as

being obvious over Haff et al., USPN 5,827,480. Applicants respectfully traverse.

The present application claims priority to the '029 (filed June 4, 1990) and '966 (filed January 2, 1992) applications, as well as U.S. Application No. 08/537,612, filed October 2, 1995. The present application is a continuation of the latter priority document and the prority documents support the present claims. Haff et al. was filed March 25, 1997 and issued October 27, 1998. Each of the cited priority documents predates the publication of the Haff refernce and, as discussed above, support the claims in question. As such, the Haff et al. reference is not prior art under 35 U.S.C. § 103(a). Therefore, the Examiner is respectfully requested to withdraw this rejection.

On the basis of the amendments and remarks presented herein, Applicants believe that this application is now in condition for immediate allowance. Applicants respectfully request that the Examiner pass this application to issue, and early notice of such is requested.

Respectfully submitted,

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APPENDIX

30. (Amended) An improved method of amplifying a nucleic acid sequence by thermal cycling of the nucleic acid sequence in the presence of a thermostable DNA polymerase, said method comprising the steps of:

(a) placing a biological sample comprising said nucleic acid sequence in a [capillary vessel] sample holding structure;

(b) raising the temperature of the biological sample from a first temperature to a second temperature wherein the second temperature is at least 15°C higher than the first temperature;

(c) holding the biological sample at the second temperature for a predetermined amount of time;

(d) lowering the temperature of the biological sample from the second temperature to at least the first temperature,

(e) holding the biological sample at a temperature at least as low as the first temperature for a pre-determined length of time;

(f) raising the temperature of the biological sample to the second temperature; and

(g) repeating steps c through f, wherein those steps are completed in approximately 10 to 90 [60] seconds [or less].

31. (Amended) The improved method of claim 30 wherein the [capillary vessel defines] sample holding structure has a volume ranging from about [10] 0.1 microliters to about [100] 10,000 microliters.

32. The improved method of claim 30 wherein the pre-determined length of time for holding step (c) is less than one second.

33. The improved method of claim 30 wherein step (d) comprises lowering the temperature of the biological sample to a third temperature that is below the first temperature, step (e) comprises holding the biological sample at the third temperature for a pre-determined length of time, said method further comprising the step of raising the temperature of the biological sample back to the first temperature and holding the sample at the first temperature for a pre-determined length of time before proceeding to step (f).

34. The improved method of claim 33 wherein the pre-determined length of time for holding step (e) is less than one second.

35. (Amended) An improved method of modifying a biological sample by subjecting the biological sample to multiple cycles of controlled rapid heating and cooling, said method comprising the steps of placing the sample in a [capillary vessel] sample holding structure and thermally cycling the sample by contacting the [capillary vessel] sample holding structure with a heated fluid to raise the temperature of the sample from a first temperature to a second temperature, cooling the sample from said second temperature to a temperature at least as low as the first temperature, and heating the sample back to the second temperature; wherein the difference between the first temperature and the second temperature is at least 15°C, and wherein the heating and cooling step are completed in approximately 10 to 60 seconds, [or less, while] such that the temperature homogeneity in [the] a 10 µL sample

during a 30 second cycle is maintained within plus or minus 1°C during the heating and cooling steps.

36. In a method of amplifying a DNA sequence by thermal cycling of the nucleic acid sequence in the presence of a thermostable DNA polymerase, wherein each cycle comprises the steps of heating a biological sample containing said DNA sequence to a denaturing temperature; holding the biological sample at the denaturing temperature; cooling the sample to an annealing temperature, holding the temperature at the annealing temperature for a pre-determined amount of time; warming the sample to an elongation temperature; and holding the sample at the elongation temperature for a predetermined amount of time; the improvement comprising limiting the holding time of the annealing and denaturing steps to less than one second each.

37. A method for improving the purity of a product produced by polymerase chain reaction wherein a DNA sequence is amplified by thermal cycling of the sequence in an aqueous sample in the presence of a thermostable polymerase and wherein each thermal cycle comprises heating the aqueous sample to a denaturation temperature, holding it at the denaturation temperature for a predetermined period of time, cooling the sample to an annealing temperature and holding it at the annealing temperature for a predetermined period of time, the method comprising the step of limiting the time the sample is held at the annealing temperature to less than 5 seconds.

38. The method of claim 37 wherein the sample is held at the annealing temperature for less than one second.

39. (Amended) The method of claim 37 further comprising the step of cooling the sample from the denaturation temperature to the annealing temperature at a rate of at least about [1.48°C] 0.5°C per second.

40. (Amended) The method of claim 37 wherein each thermal cycle is completed in [less than] 10 to 60 seconds.

41. The method of claim 37 wherein the period of time the sample is held at the denaturation temperature during each thermal cycle is less than 32 seconds.

42. The method of claim 41 wherein the period of time the sample is held at the denaturation temperature during each thermal cycle is less than one second.

43. A method for increasing the yield of an amplified DNA sequence by use of polymerase chain reaction wherein the DNA sequence is thermally cycled in an aqueous sample in the presence of a thermostable polymerase and wherein during each thermal cycle the sample is heated to a denaturation temperature and held for a predetermined period of time, said method comprising the step of limiting the time the sample is held at the denaturation temperature to less than 32 seconds.

44. The method of claim 43 wherein the sample is held at the denaturation temperature for less than one second.

45. (Amended) The method of claim 43 wherein each thermal cycle is completed in [less than] approximately 10 to 60 seconds.